REMARKS

Claim 41 is newly presented. Claims 39 and 40 are canceled. Claims 17-18, 21-38 and 41 are currently pending in the application. Claims 17, 18, 21, 22, 23, 24, 27, 34, 35 and 36 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Claim Objections

New Claims 39 and 40 depend from claims 22 and 23, respectively and are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim.

Applicants submit that claims 39 and 40 are currently canceled, rendering the objections of the previous Office Action moot.

Claim Rejections -35 USC § 112

Claims 17, 24 and 27 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Office Action states that Claims 39 and 40 recite the term "proteolysis", this term lacks antecedent basis in claims 22 and 23 from which claims 39 and 40 depend.

As previously mentioned, Applicants have canceled claims 39 and 40. As such, Applicants submit that the 35 U.S.C. §112 rejection of these claims has been rendered moot.

The Office Action further states that Claim 17 is directed to a polypeptide pair that can be, or is not covalently modified through the recitation of "and the reversal of these covalent modifications". The Examiner asserts:

> "this phrase is a conditional phrase, based upon a reversal act, but if the covalent modification that is "required for said association" is reversed, then the polypeptide pair may not be "bound to" each other. The claims recite a contradictory combination of claim limitations that is not internally consistent with what is being claimed. How can both conditions be true at the same time, specifically, a complex formed from the first and second polypeptides ONLY when one of them is covalently modified, as well as being a complex which is NOT covalently modified. The combination of claim limitations is confusing as

> to what is actually present or absent from the claimed pair is not clearly set forth in the claim."

Applicants have amended claim 17 to recite a polypeptide pair ". . . wherein said covalent modification comprises one of the group consisting of phosphorylation, dephosphorylation. acylation, deacylation, glycosylation, deglycosylation, ubiquitination, deubiquitination, prenylation, deprenylation, sentrinization, desentrinization, ADP-ribosylation and ADP-deribosylation." Applicants submit that support for dephosphorylation, deubiquitination, deprenylation, desentrinization and ADP-deribosylation are drawn from the recitation within the specification, "or the reversal thereof." Applicants submit that claim 17, as currently amended, no longer recites "and the reversal of these covalent modifications," and clearly sets forth the types of modifications required for the polypeptide binding partners to bind each other. Therefore, Applicants submit that claim 17, as currently amended, is definite, and respectfully request withdrawal of the 35 U.S.C. §112, second paragraph rejection and reconsideration of the instant claim.

The Office Action states that no radioactive or fluorescent labels are recited or provided in claims 22 or 23, and that "Claims 24 and 27 should recite --- further comprising a radioactively or fluroescently labeled polypeptides --- or --- wherein the measuring is through a radioactive or fluorescent label on the first or second polypeptide ----; or an equivalent phrase." The Examiner further asks:

> "[i]sn't the "modification" a type of label when a change in molecular mass is measured; claims 24 and 27 are not further limiting of claims 22-23 through the broad recitation of any type of label, when the "modification" set forth in the independent claims are a type of label that permits the detection or measuring of the modification through molecular mass measurements (see claims 31-32, which depend from claims 22-23). Clarification of the type of label, and distinguishing the "modification" from the presence of an additional label would define claims 24 and 27 as being further limiting of claims 22 and 23."

As suggested by the Examiner, Applicants have currently amended claims 24 and 27 to recite ". . . further comrpise[s] a label" in order to distinguish the label in the instant claims from the covalent modification. Therefore, Applicants respectfully request withdrawal of the

withdrawal of the 35 U.S.C. §112, second paragraph rejection and reconsideration of the instant claims.

Claim Rejections -35 USC § 102

Claim 17 is rejected under 35 U.S.C. 102(b) as being anticipated by Fitzpatrick et al (US Pat. 5,710,009). The Office Action states that Fitzpatrick et al disclose and claim a composition that comprises:

> an immobilized complex (see claim 19) that comprises the combination of first and second polypeptides, specifically a peptide "reland" (col. 5, lines 4-6, line 22) together with its receptor (see col. 5, lines 54-60).

The Office Action further states that reference anticipates the instantly claimed complex that comprises first and second polypeptides that does not comprise a covalent modification based upon the fact that it is not a required component of the instantly claimed polypeptide pair through the recitation of the phrase "and the reversal of these covalent modifications" which defines the scope of the claimed invention to not require the presence of the covalent modification.

With this amendment, Applicants have amended claim 17 to recite:

17. A polypeptide pair comprising a first polypeptide immobilized to a support, and a second binding partner polypeptide bound to the first polypeptide, wherein the binding of the polypeptides to each other is detectable, and covalent modification of at least one of the polypeptides results in modulation of the binding, and is required for said binding of said first polypeptide and said second binding partner polypeptide, wherein said second binding partner polypeptide is not a phospho-specific antibody, and wherein said covalent modification comprises one of the group consisting of phosphorylation, dephosphorylation, acylation, deacylation, glycosylation, deglycosylation, ubiquitination, deubiquitination, prenylation, deprenylation, sentrinization, desentrinization, ADP-ribosylation and ADP-deribosylation.

As amended, claim 17 no longer recites the phrase "and the reversal of these covalent modifications," but rather recites that the modification be "selected from the group consisting of phosphorylation, **dephosphorylation**. acylation, **deacylation**, glycosylation, **deglycosylation**, ubiquitination, **deubiquitination**, prenylation, **deprenylation**, sentrinization, **desentrinization**, ADP-ribosylation and **ADP-deribosylation**." (emphasis added) As previously discussed, support for dephosphorylation, deacylation, deglycosylation, deubiquitination deprenylation, desentrinization and ADP-deribosylation are drawn from recitation of the phrase "and the

reversal of these covalent modifications." Applicants further submit that all the recited modifications of the instant claim require covalent modification. As such, Applicants submit that claim 17, as currently amended, is novel over Fitzpatrick, and respectfully request withdrawal of the §102(b) rejection over this reference.

Claim 17 is rejected under 35 U.S.C. 102(b) as being anticipated by Hochstrasser et al (US Pat. 5,565,352). The Office Action states:

> "Hochstrasser et al disclose a composition that comprises:
>
> an immobilized complex (see Figure 6a), the complex comprising the combination of first and second polypeptides (ubiquitin-oligopeptide covalent conjugate (see col. 1, lines 23-25, and lines 26-45). immobilized on a solid immunoblot surface (see Figures 6a and 6b), immunoreacted with an anti-ubiquitin antibody polypeptide; also see col. 6, lines 63-67 and col. 7, lines 1-16; Example 4, col. 40, lines 14-56).
>
> Figure 6b shows the combination of polypeptide substrate that has been ubiquintinated with a covalent bond to a ubiquintin polypeptide (see complex formed between "I" and "II" which is subsequently combined with an additional polypeptide "Doa4" in step "IV", the association of which would not take place without the covalent modification of one of the polypeptides with ubiquitin. The product of the Doa4/polypeptide complex is deubiquitin polypeptides ("peptides").
>
> The reference anticipates the instantly claimed complex that comprises first and second polypeptides that comprises a covalent modification, as well as a complex that does not require a covalent modification of one of the polypeptides (ubiquitin/antibody complex)."

Applicants respectfully disagree, particularly with the Examiner's assertion that a ubiquitin-oligopeptide covalent conjugate, as taught by Hochstrasser et al., is within the scope of a first and second polypeptide of the instant claim. Applicants submit that claim 17, as amended herein, recites first and second polypeptides "bound" to each other. As discussed previously, Applicants submit that claim 17 has been amended such that it no longer recites the phrase "and the reversal of these modifications," and further submit that all the recited modifications are covalent modifications. Applicants submit that the term "binds" has been defined in the Specification to be synonymous with "associates" as follows:

> [T]he term "associates" or "binds" refers to polypeptides as described herein having a binding constant sufficiently strong to allow detection of binding by a detection means, such as FRET or surface plasmon resonance. Preferably, the polypeptides, when

> associated or bound, are in physical contact with each other and have a dissociation constant (Kd) of about 10μM or lower. (Page 10, last paragraph)

Therefore, "associates," "binds" and derivatives thereof are defined as having a binding constant. A "ubiquitin-oligopeptide covalent conjugate," as taught by the Hochstrasser et al., is a **covalent** conjugate and therefore lacks a binding constant. As such, Applicants submit that Hochstrasser et al. does not meet the limitation of the invention as claimed, as it does not teach a "polypeptide pair comprising a first polypeptide immobilized to a support, and a second binding partner polypeptide **bound** to the first polypeptide," (emphasis added) as the term 'bound' is defined in the instant application. Therefore, Applicants submit that Hochstrasser et al. does not anticipate claim 17. As such, Applicants respectfully request withdrawal of the 102(b) rejection over this reference and reconsideration of claim 17.

Claims 18, 21-26, 31, 34-40 are rejected under 35 U.S.C. 102(b) as being anticipated by Hochstrasser et al (US Pat. 5,565,352). The Office Action states:

> Hochstrasser et al disclose the instantly claimed method of detecting or monitoring the activity of a modifying agent in the presence of candidate compound; a method of detecting a modifying enzyme on a modifying enzyme that comprises the steps of: Providing first immobilized (col. 29, lines 40-44; E2 enzyme or deubiquination enzyme and/or ubiquitin, see col. 1, lines 22-25; col. 33, lines 53-59; first antibody affixed to a solid support (col. 4, lines 34-35; col. 32, lines 56-57); or "enzyme coupled to a solid support (see col. 26, lines 66-67 and col. 27, lines 1-5); or affixed to solid support (see col. 29, lines 30-43 and col. 29, lines 5-14); col. 33, line 16 "attached to solid support") and second (short lived eukaryotic protein; a type of polypeptide, col. 1, lines 22-25 and col. 1, lines 45-60) polypeptides,
>
> wherein at least one of the polypeptides is susceptible to modification (deubiquination (col. 1, lines 45-47; col. 7, lines 9-16); or ubiquitination, col. 33, lines 55-65 "E2 modifying enzymes") and the two polypeptides are capable of binding to each other (see col. 14, line 7);
> and covalent modification (see col. 33, lines 29-51) of one or both of the polypeptides by the modifying agent (ubiquitination is a covalent modification "E2 enzymes, col. 33, lines 55-65 and col. 34, lines 3-8; or the modification may be glycoylation or prenylation (see col. 26, lines 27-3 3) or deubiquination), in the presence of a modifying group substrate (see col. 29, lines 13-15; figure 6b) results in modulation of the binding of the polypeptides to each other (see Figure 6b, binding is modified to comprise an additional covalent modification, or release of a polypeptide; Figure 6b shows the combination of polypeptide substrate that has been ubiquintinated with a covalent bond to a ubiquintin polypeptide (see complex formed between "I" and "II" which is subsequently combined with an additional polypeptide "Doa4" in step "IV", the association of which would not

take place without the covalent modification of one of the polypeptides with ubiquitin.
The product of the Doa4/polypeptide complex is deubiquitin polypeptides ("peptides")).
Allowing the polypeptides to bind to each other (see at least Figure 6b).
Contacting the polypeptides with a modifying agent (E2 enzyme or deubiquintinating enzyme or agonist or antagonist (see col. 27-28) in the presence of said modifying group substrate (see col. 27, lines 19; forms ubiquitin-oligopeptide covalent conjugate (see col. 1, lines 23-25, and lines 26-45) or removes ubiquitin from the protein ubiquitin conjugate (see col. 14, lines 4-20); introduces or removes glycosylation or prenylation groups (see col. 26, lines 30-34);
Detecting modulation (see col. 28, lines 54-59; col. 28, lines 24-40) of the binding of the polypeptides to determine a reference signal (baseline activity, see col. 27, lines 19-20) modulation (see col. 25, lines 37-50; section IX, col. 25-28)
Contacting the polypeptides with a modifying agent (enzyme that modifies the first and second polypeptide complex) and a candidate modulator (see col. 26, lines 14-42; col. 27, lines 14-57) of the modifying agent; and
Detecting modulation of binding of the polypeptides in the presence of said candidate modulator and comparing the modulation detected (see col. 28, lines 16-67 in the presence of said candidate modulator with the reference signal ("a control" see col. 28, lines 66-67; col. 29, lines 1-20; "baseline", see col. 27, lines 18-27) modulation (see col. 27, lines 14-17; col. 25, lines 37-50; col. 26, lines 13-43).

Applicants respectfully disagree. As previously discussed, Applicants submit that Hochstrasser et al. does not teach a "polypeptide pair comprising a first polypeptide immobilized to a support, and a second binding partner polypeptide **bound** to the first polypeptide," (emphasis added) as the term 'bind' or 'bound' is defined in the instant application. Therefore, Applicants submit that ubiquitin cannot be considered one of the first or second polypeptides according to the present invention, since ubiquitin is covalently attached to the other polypeptide and is outside the scope of 'bound' as defined in the Specification in this context.

Applicants further submit that the instant claims require the presence of the following distinct elements:

- a first polypeptide (independent claims 18, 21, 22, 23, 34, 35);
- a second polypeptide (independent claims 18, 21, 22, 23, 34, 35);
- a modifying enzyme (independent claim 18; detecting in 21, 22, 23, 34, 35); and
- a modifying group substrate (independent claims 18, 21, 22, 34, 35)

Applicants submit that if the E2 enzyme or deubiquitinating enzyme is to be considered a first immobilized polypeptide as the Examiner asserts, then these enzymes cannot be considered a modifying enzyme; Hochstrasser would therefore fail to teach a modifying enzyme or a modifying group substrate if E2 or the deubiquitinating enzyme is the first polypeptide.

Alternatively, if the E2 or a deubiquitinating enzyme is to be considered the modifying enzyme, it cannot be simultaneously considered the first or second polypeptide. Applicants further submit that claims 21, 22, 23, 34 and 35 recite methods for detecting the presence of a modifying enzyme in a sample. As such, Applicants submit that, in claims 21, 22, 23, 34 and 35, the designation of E2 enzyme or deubiquitinating enzyme as either the first or second polypeptide recited in the claim would require the method to detect another enzyme activity to meet all the elements of the claim. In other words, how does one detect the presence of a modifying enzyme in a sample if the enzyme is already required to be present in the assay mixture? Applicants therefore submit that Hochstrasser fails to provide all the elements of independent claims 18, 21, 22, 23, 34 and 35, as well as claims 24, 25, 26, 31, 36 and 40 which depend from these claims. As such, Applicants respectfully request withdrawal of the 102(b) rejection and reconsideration of these claims.

Claims 18, 21-33, 36-40 are rejected under 35 U.S.C. 102(e) as being anticipated by Wagner et al (US Pat. 6,475,809, effective filing date July 14, 1998). The Office Action states:

> "Wagner et al disclose . . . a method of detecting a modifying enzyme on a modifying enzyme that comprises the steps of:
> **Providing** first immobilized and second polypeptides (see col. 7, lines 40-55 "ras-like GTPases", kinases, phosphatases, hydrolases, proteases, proteases; col. 17, lines 35-41 (enzyme/substrate); all claims), wherein at least one of the polypeptides is susceptible to modification (see Example 7, and col. 17, lines 35-57) and the two polypeptides are capable of binding to each other (see claims and Example 7);
> and covalent modification (see col. 33, lines 29-51) of one or both of the polypeptides by the modifying agent (see col. 5, lines 51-60; col. 7, lines 40-55), in the presence of a modifying group substrate results in modulation of the binding of the polypeptides to each other (see all claims)
> Allowing the polypeptides to bind to each other (see Example 7, and claims; col. 17, lines 22-57)
> **Contacting** the polypeptides with a modifying agent (see Example 7, and claims, col. 18, lines 6-41) in the presence of said modifying group substrate;
> **Detecting** modulation (see Example 7, and FRET detection methods (two labels, col. 17, lines 47-57) of the binding of the polypeptides to determine a reference signal (baseline activity, see col. 27, lines 19-20) modulation (see col. 25, lines 37-50; section IX, col. 25-28)
> Contacting the polypeptides with a modifying agent and a candidate modulator (see Example 7 and col. 17, lines 35-41 and claims) of the modifying agent; and
> Detecting modulation of binding of the polypeptides in the presence of said candidate modulator (drug candidate, col. 18, lines 15-19) and comparing the modulation detected

in the presence of said candidate modulator modulation (see Example 7 and col. 18, lines 6-23)
(Instant claim 24-25, 27-30) at least one of the polypeptides is labeled ("Fret", more than one label (see col. 17, lines 47-57, and thus use of a label: Example 7).
(Instant claim 26) wherein the label is radioactive (see col. 22, lines 43-45).
(Instant claim 31-33) measured by monitoring molecular mass ("NMR" see col. 22, Example 3) and (surface plasmons, col. 17, line 55; chemiluminescence's, surface charge sensors). The reference anticipates the instantly claimed invention."

With this Amendment, Applicants have amended the instant claims to recite "modifying enzyme," rather than "modifying agent." Applicants therefore submit that Wagner et al. do not teach or suggest covalent modification by a modification enzyme, as is recited in the instant claims. Rather, Wagner et al. disclose the covalent attachment of an adaptor molecule to an affinity tag or protein or both (via chemical conjugation or as a fusion protein, for instance), or the immobilization of various proteins on an array (col. 7, lines 40-55).

Regarding covalent modification of polypeptides, Applicants submit that column 33, which the previous Office Action pointed to for the presence of support for covalent modification, is not present in Wagner et al., and request clarification. Applicants further submit that Wagner et al. fail to disclose a covalent modification that results in modulation of the binding of polypeptides to each other. Wagner et al. do not teach or suggest a "covalent modification of one or both of the polypeptides by the modification enzyme in the presence of a modifying group substrate results in modulation of the binding of the polypeptides to each other," as is recited by claim 18, or "wherein covalent modification of said first polypeptide detectably changes the association of said first and second polypeptide," as is recited in claim 21. Therefore, Applicants submit that Wagner et al. do not anticipate the claims of the present invention. As such, Applicants respectfully request withdrawal of the 102(e) rejection of claims 18, 21-33, 36-40 over Wagner et al., and reconsideration of the instant claims.

Claims 22-23, 39-40 (proteolysis species) are rejected under 35 U.S.C. 102(e) as being anticipated by Shone et al (U.S. Pat. No. 5,962,637, filing date December 3, 1996). The Office Action states that "Shone et al disclose a method of detecting the presence of a modifying enzyme in sample, wherein the enzyme modifies a polypeptide covalently, and the enzyme is

botulism or tetanus toxin." The Office Action further states that the method comprises the steps of:

> Providing a polypeptide pair, a first polypeptide and a binding partner (see Shone et al, claim 1), the first polypeptide is a substrate polypeptide and the binding partner is an antibody capable of binding the substrate with a covalent modification with the enzyme and is labeled with an enzyme;
> providing and immobilizing the first polypeptide to a physical support (see Shone et al claim 1, attached to solid support, the immobilized polypeptide is labeled with the detectably labeled antibody to form a detectable complex upon covalent modification with the enzyme in a sample; contacting the immobilized polypeptide with the second polypeptide (test compound, see Shone et al claims 1, 2 and contacting the immobilized polypeptide with said binding partner polypeptide (see claims 1 and 9, Shone et al) Measuring (assaying) the modification (cleavage of substrate) of at least one of the polypeptides by measuring the association of the binding partner polypeptide (antibody binds, see Shone et al The firs polypeptide contains a detectable label (see Shone et al, claims 6-7, detection of label). An antibody-binding partner linked to an enzyme is also taught to include a radioactive label (see Shone et al col. 6, lines 37-39) or a fluorescent label (see Shone et al, col. 6, lines 39-42).
> The antibody was modified by linking an enzyme to the antibody (see Shone claim 6), or an antibody that binds to the binding partner antibody that is linked to an enzyme (see Shone et al, claim 1.
> The assay also comprises the addition of a protease, an enzyme with proteolytic activity (Instant claims 39 and 40) to the test compound which is capable of functioning as an agent (see Shone et al, claim 1, an enzyme that can covalently
> modify a polypeptide, such as activate an inactive endopeptidase.)
> One polypeptide is immobilized to a support and a second polypeptide bound to the first polypeptide, wherein the two polypeptides are an endopeptidase peptide substrate linked to a carrier protein that is maleimide activated BSA, which is bound to a microtiter plate (see Shone et al col. 15). An additional embodiment disclosed is a polypeptide pair comprising a first lines 45-55. The binding of the two polypeptide one to the other is detectable, and a covalent modification of one of the polypeptides is required for the association of the first polypeptide endopeptidase substrate with the binding partner polypeptide. The assay is carried out over time, thus defining an assay carried out in real time. The reference anticipates the instantly claimed invention.

Applicants respectfully disagree. As previously stated, Applicants submit that claims 39 and 40 are currently canceled. Further, Applicants submit that Shone et al. does not teach a covalent modification selected from the group recited in claims 22 and 23 as currently amended, since the covalent modification in claims 22 and 23 do not encompass proteolysis but are limited to the group consisting of phosphorylation, acylation, glycosylation, ubiquitination, prenylation, sentrinization, and ADP-ribosylation. As such, Applicants submit that Shone et al. does not

anticipate the instant claims. Therefore, Applicanats respectfully request withdrawal of the 102(e) rejection over this reference and reconsideration of claims 22-23, 39 and 40.

With this Amendment, Applicants have made an earnest effort to respond to all issues raised in the Office Action of August 5, 2004, and to place all claims presented in condition for allowance. Applicants submit that in view of the preceding remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over the claims of the present invention. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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Barbara A. Dyne (34,614) for Kathleen Williams

Name: Kathleen M. Williams
Registration No.: 34,380
Customer No.: 29933
Palmer & Dodge LLP
111 Huntington Avenue
Boston, MA 02199-7613
Tel: 617-239-0100